Lab 7: Brownian Motion

I. Before you come to lab...

A. Read the handout you picked up at Thursday's lecture on viscosity and Brownian motion.

B. Review the background material on statistics and Gaussian distributions from Lab 1.

C. Play around with the random walk applet at the following URL:
http://www.rpi.edu/dept/materials/MEG/Java_Modules_files/RandomWalk/RandomWalkApplet.html
(There is also a link to it from the PS2 website.)

D. Read this handout in its entirety.

E. Complete the pre-lab assignment at the end of this handout.

II. Background

A. A little bit more on statistics

1. Standard deviation

Earlier in the semester, we told you that standard deviation σ is a number which characterizes the "spread" in a distribution or data set. Let's take a closer look at how the standard deviation is calculated.

a. The standard deviation is defined as the **RMS deviation from the mean**. RMS stands for "root mean square." What this definition means is that in order to calculate σ, you first look at every data point in the distribution and figure out how far it is from the mean. Then you square that distance, and average over all the data points, and finally take the square root.

b. In equation form, if μ is the mean of the distribution of x_i's, then

$$\sigma = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - \mu)^2}$$

Alternatively, using the angle bracket notation for mean or average,

$$\sigma = \sqrt{\left\langle \left(x - \langle x \rangle\right)^2 \right\rangle}$$

This definition will be useful to us when we consider the distribution of steps in a random walk (Brownian motion).

2. Uncertainty of the standard deviation

a. You might recall that for a data set with N measurements, we can use the mean of the data set as a *best estimate* of the "true" mean of the underlying distribution. The uncertainty of this estimate is given by something called the *standard error of the mean*, which is σ/√N.

b. In the same way, the standard deviation of the set of N measurements is our best estimate of the "true" standard deviation of the underlying distribution. As with estimating the mean, this estimate has an uncertainty, which is called the *standard error of the standard deviation*.

(1) Ordinarily, we don't really care that much how accurate our estimate of σ is, because we are only using σ to characterize the uncertainty of something else (the mean), and the uncertainty of an uncertainty is not usually of much interest.

(2) However, there are instances in which σ itself is the measured value of some quantity, in which case we would very much like to know the uncertainty in our estimate of σ. This is where the standard error of σ comes in:

$$\mathrm{SE}(\sigma) = \frac{\sigma}{\sqrt{2N-2}}$$

B. Random walks: the quick & dirty summary

Recall the basic premise for a random walk: every τ seconds, you flip a coin. If it's heads, you move to the right by a distance δ; if it's tails, you move to the left by δ. Here are the results we derived in class:

1. In one dimension

a. Any single random walk is fundamentally not predictable, and also not very informative. It's only when you **average over many random walks** that meaningful quantitative trends start to emerge.

b.

$$\langle x \rangle = 0$$

That is, **on average, you don't go anywhere** (either to the left or to the right), no matter how many steps you take.

c. Now we can directly apply all of the results from our random walk model, using a step size of δ and a time between steps of τ. For one-dimensional Brownian motion, the result is:

$$\langle x^2 \rangle = 2Dt$$

That is to say, **you do go somewhere** (away from where you started), but **the average squared distance from the starting point only increases linearly as the number of steps**. It doesn't take long to get a few steps away, but if you want to go twice as far you have to wait four times as long. The *diffusion constant* D is defined by

$$D = \frac{\delta^2}{2\tau}$$

The factor of 2 in the denominator is there for convenience.

2. In more dimensions

a. x, y, and z each independently obey both of the equations for random walks in one dimension. So it remains true that no matter how many steps you take, on average you will move neither left nor right, neither forward nor backward, and neither up nor down.

b. However, r^2, the distance from the origin, is expected to increase with the number of steps. Since $r^2 = x^2 + y^2 + z^2$, we can write $\langle r^2 \rangle = 6Dt$ in 3 dimensions, or $\langle r^2 \rangle = 4Dt$ in 2 dimensions.

C. Brownian motion

1. Surprisingly, the simple random walk is a very good model for Brownian motion: a particle in a fluid is frequently being "bumped" by nearby molecules, and the result is that every τ seconds, it gets jostled in one direction or another by a distance δ. You could make the model more sophisticated, but this very simple model has all of the important (and correct) features that a more complete analysis would provide.

2. The bottom line is, we can still use the important quantitative result that $\langle x^2 \rangle$ increases as 2Dt (in one dimension). This means that if we actually perform a Brownian motion experiment and measure the average squared displacement in a certain time interval, we can determine the diffusion constant D.

3. For 2- and 3-dimensional Brownian motion, the same equation holds for each of x, y, and z independently. So for example, in two dimensions, the mean squared displacement from the origin is equal to 4Dt.

4. In general, D depends on the size and shape of the diffusing particle, as well as on the temperature. In 1905 Einstein, by thinking about viscous drag and thermal energy, derived the important relationship:

$$Df = k_B T$$

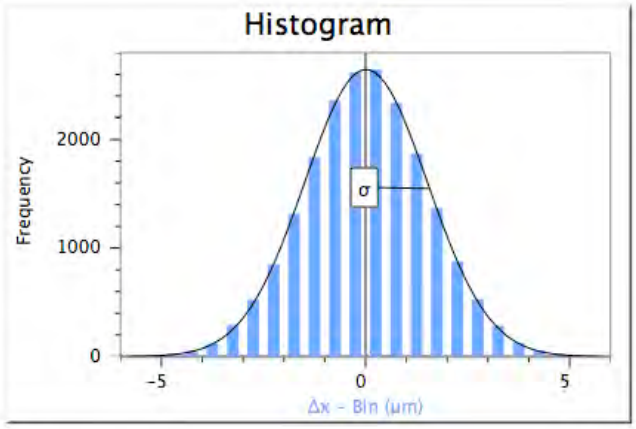
This is known as the *Einstein-Smoluchowski* equation. The constant f on the left-hand side is called the *drag coefficient*; it is the proportionality constant between the viscous drag force on the particle and its speed, i.e. $F_{drag} = fv$. Recall that for a sphere of radius r, the Stokes formula gives $f = 6\pi\eta r$.

5. Because D, f, and T are easily measurable experimentally, the Einstein-Smoluchowski equation gave the first way of making a direct measurement of Boltzmann's constant k_B. And since Boltzmann's constant is just the ideal gas constant (which had been known for over a century) divided by Avogadro's number, this was one of the first

measurements of Avogadro's number, and a convincing proof of the theory that matter is made of discrete molecules.

6. Remember that in order to measure the **mean** (or average) squared displacement, you need to perform **many random walks**, or equivalently, many Brownian motion experiments. A single random walk won't necessarily resemble the average at all. There are two basic ways of "repeating" a Brownian motion experiment:

a. If you have a large number of particles all at the same starting point, and take a snapshot of where they all end up at a later time, then each particle has undergone an independent random walk. Averaging the values of x^2 for each particle would enable you to determine D. Even better, if you plotted a histogram of the x values (not squared) for each particle, you'd actually see ... our old friend, the Gaussian distribution:



(We'll learn why this is the case when we study the diffusion equation.) The mean of this Gaussian is the average displacement, which is zero. The standard deviation σ is just the RMS displacement, so $\sigma^2 = 2Dt$ (in one dimension).

b. If you take a single particle in Brownian motion and measure its position many times at regular intervals, you are effectively performing many short random walks in succession. The key, however, is that each time you measure its position, what you are really interested in is *how far it has moved since the last time you measured it*, not on how far it has moved since its initial position. That way each random walk will have the same (short) time t, so you can figure out the average x^2 during that time t and thus measure D.

III. Introduction

A. In this lab, you'll explore Brownian motion. You'll observe a micron-sized sphere under a microscope, and watch as it undergoes a random walk in two dimensions. You'll then quantitatively analyze its motion and measure its diffusion constant. Using known properties of the sphere, you can then experimentally determine Boltzmann's constant and Avogadro's number, just as Einstein and Perrin did in the early 20th century. Finally, you'll get a chance to observe motion that is *not* Brownian, but rather due to self-propelled micro-organisms.

B. Objectives for this lab:

1. Directly observe Brownian motion under a microscope

2. Measure the diffusion constant for a 1-micron sphere in water

3. Experimentally determine Boltzmann's constant

4. See how the motion of self-propelled organisms differs from Brownian motion

IV. Materials

A. 1 microscope

1. There are two types of microscopes: the grey Bausch & Lomb microscopes, and the black Spencer microscopes. They are largely equivalent.

Bausch & Lomb Spencer


2. The microscopes have been fitted with an LED light source and a modified iSight camera. The microscope objective will cast an image of the sample on the slide directly onto the CCD array of the camera; illumination is provided from below the microscope stage by the LED. From this setup, Logger Pro can capture time-lapse video of Brownian motion of particles suspended in solution on the microscope slide.
3. Each microscope has an objective for 10X magnification, and an objective for approximately 40X magnification. (Some of the microscopes may have 43X instead of 40X.)
4. Each microscope also has two focus knobs. The larger/upper knob is for coarse focus adjustments, and the lower one is for fine adjustments.
5. The on/off switch for the LED is located just below the 9-volt battery.

B. Microscope slides

1. 
2. The slides are well slides, which means they have a slight depression in the center to hold the sample.

C. Cover slips

D. Microsphere solution

1. This is a solution of micron-sized polystyrene spheres in water. You'll place a drop of this solution onto the well slides and then observe it under a microscope.
2. Diameter of microsphere = (1.025 ± 0.010) µm
3. Viscosity of microsphere solution = (9.5 ± 0.5) x 10^{-4} Pa s

V. Procedure

A. Take a photo of yourselves...

1. This time, because the external iSight cameras are being used for microscopy, you will need to convince Photo Booth to use the built-in camera above the monitor. To do this:
   - **a.** Open the file Lab7.cmbl in Logger Pro.
   - **b.** From the Insert menu, select Video Capture...
   - **c.** When it prompts you for which camera source to use, choose IIDC FireWire (which is the external iSight camera).
   - **d.** If it asks you for a resolution, select 800x600. If it asks you for the audio source, pick any of the options.
   - **e.** Now open Photo Booth. Because Logger Pro is using the external camera, Photo Booth will automatically default

to using the built-in camera. Take a picture of your lab group and drag it into the space below:

2. Tell us your names:
- **a.**
- **b.**
- **c.**

B. Measure the Brownian motion of a 1-micron polystyrene sphere in water.

1. Familiarize yourself with your microscope.

2. Prepare a sample.
- **a.** A sample preparation area is next to the sink. There are several bottles of 1-micron spheres (as well as various biological samples).
- **b.** Using the pipette, place a drop or two of solution into one of the well slides, and cover it with a cover slip. Make sure not to trap air bubbles under the cover slip.
- **c.** Invert the slide onto a Kimwipe and tap it gently to remove excess solution.
- **d.** After a few minutes the edges will dry, and the cover slip will become lightly stuck to the well slide. This should provide a sample with minimal evaporation. Evaporation would cause an overall drift of particles in the direction of the fastest evaporation, which is something we don't want.
- **e.** To further reduce evaporation, gently paint the edges of the cover slip using clear nail polish. This will provide an even tighter seal on the solution in the well.

3. Observe the sample under the microscope.
- **a.** Load the sample into the microscope by clipping the slide onto the microscope stage.
- **b.** Go to Logger Pro. The Video Capture window should already be open.
- **c.** Focus an image onto the camera. Use the 10X objective first, and learn how to focus on spheres throughout the depth of your sample.
- **d.** Switch to the 40X objective, which you will use to collect data, and master the more delicate task of focusing on spheres at this higher magnification. Be careful! On the 40X objective, it is very possible to break your slide and/or cover slip by trying to move the objective too close to the sample using the coarse focus knob.
- **e.** Observe the microspheres for long enough to convince yourself that the particles are diffusing with minimal overall drift. The solution should be dense enough that a few spheres are within the field of view.

4. Collect a video capture of the Brownian motion of the spheres.
- **a.** In the Video Capture window, click on the Options button. Set the following options:
  - **(1)** Video Capture Only
  - **(2)** Capture Duration: 300 seconds
  - **(3)** Time-Lapse Capture
  - **(4)** Capture Interval: 5 seconds
  - **(5)** Capture File Name Starts With: Brownian
  - **(6)** Click on OK.
- **b.** Click on Start Time Lapse. (Time Lapse differs from a regular video capture in that instead of recording continuous video, it records only one frame every 5 seconds.)
- **c.** Wait for 5 minutes. Every 5 seconds, the video will appear to freeze momentarily while Logger Pro records a frame.
- **d.** When it is finished, the captured video will appear behind the Video Capture window. Close the Video Capture window.
- **e.** Save your Logger Pro file.

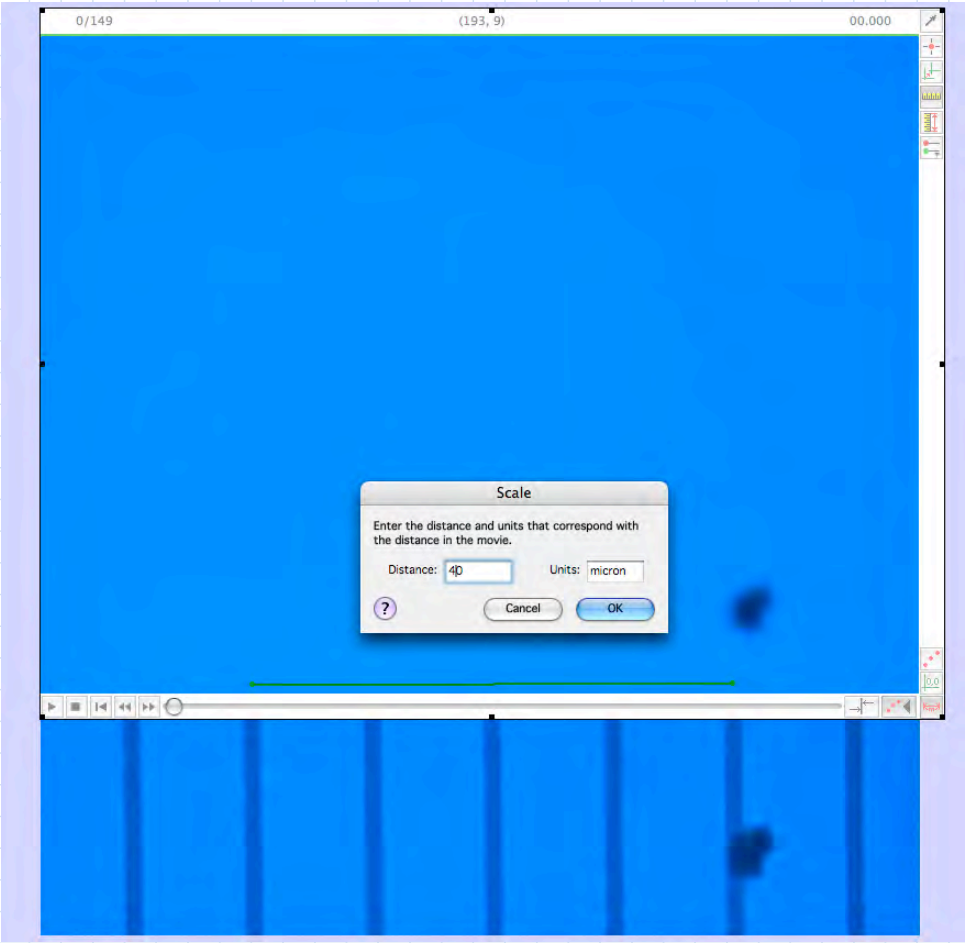
5. Set the scale for your captured video.
- **a.** From the Logger Pro Insert menu, select Picture → Picture Only.
- **b.** On your computer's desktop are two photos of a scale with 10 micron divisions, taken at 10X and 40X on your microscope. Select the appropriate photo to include on your LoggerPro page.
- **c.** Re-position the picture underneath and in line with the time-lapse video, but overlapping slightly so that you can still see the scale markings when the video window is on top.

d. Click on the  icon to bring up the video analysis functions. Click the  button to set the length scale.

e. Use the scale markings in the adjacent photo to give you a length reference, but click and drag in the video window (not the picture window) to set the scale. Enter the scale in microns (you can use the abbreviation "um" if you like).

f. The distance between the small divisions is 10 microns, hence the distance between the large divisions is 50 microns. As a point of reference, the entire window should be something like 70 or 80 microns across on 40X magnification. When you have finished setting the scale, the screen should look something like this:

(1)



6. Quickly scroll through the entire movie (by dragging the circle through the scroll bar) to see if there is a microsphere that appears to stay in the picture for the duration of the capture. This is preferable for the video analysis; however, if there is no such sphere, that's okay too. The other, and more important, thing to check for, is that there is minimal overall drift of the entire sample in the same direction. If there is significant drift, it is probably best to wait a few minutes and try again. If that doesn't work, just make a new sample.

7. Add points to your video analysis.

a. Choose a sphere that you are going to follow for the 5 minutes. Again, it is preferable but not essential to choose one that stays in view the whole time. However, make sure to choose a sphere that appears to be moving freely, rather than one that is stuck to the glass (or is perhaps not a microsphere at all, but just a smudge on the microscope or camera lens!).

b. Mark its position at every frame in the video using the  Add Point button.

c. If no single microsphere is visible for the entire video, just switch to a new one part way through. Since only the *differences* in position between adjacent points are used in the analysis, you will only lose one data point;

however, be sure to remember that you did this so you can strike out the phony data point later when you do your analysis. (It will end up looking as if the microsphere jumped all the way across the screen in a single 5-second interval.)

8. Analyze the data.

a. Make a graph of Y position vs X position.

(1) You can use your existing graph of X and Y vs time. Just click on where it says Time on the horizontal axis and change it to X.

(2) Then click on where it says "X Y" on the vertical axis and change it to just Y.

(3) Double-click on the graph and check the box for Connect Points.

(4) You should now see essentially the path traced by your sphere as it executed its five minute random walk. Paste a copy here:

b. Move to page 2 in the Logger Pro file. You should see a data table with columns for X, Y, X velocity, and Y velocity.

c. In your Video Analysis data set, define a calculated column labeled "Delta x" to give the diifference in X position between adjacent times.

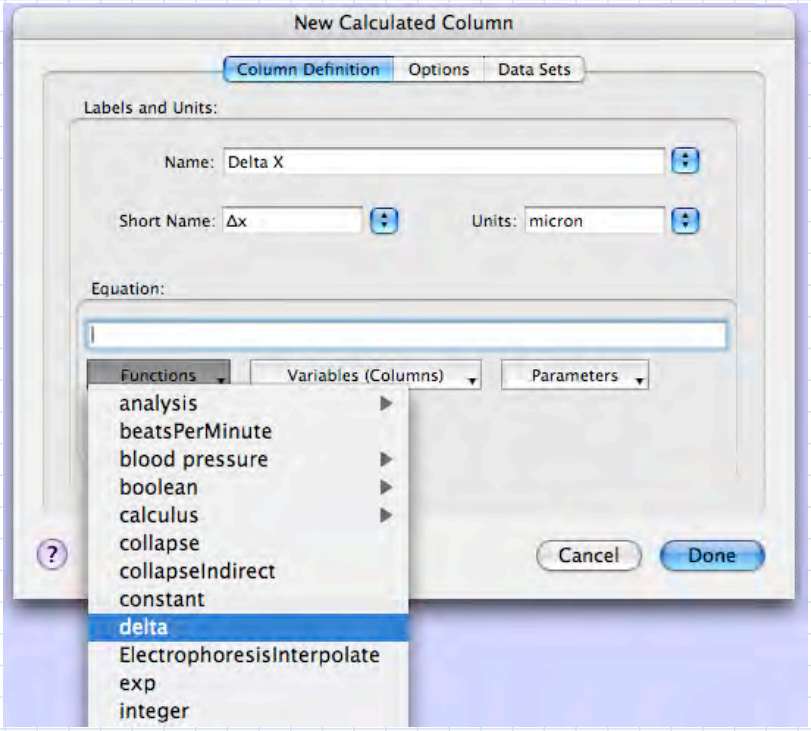
(1) Double-click on the column heading for X velocity. We won't need the X velocity, so we'll just change this column to suit our purposes better. Rename it "Delta x" and change the units from microns per second to just microns. If you want to give it a short name, too, feel free to do that.

(2) In the equation for column definition, where it says

derivative("X")

replace it with the "delta" function applied to the "X" column.

(a)



(3) This new column will be one row shorter than the X column, and will show you the change in X during each 5-second interval.

(4) Change Y velocity into a column of deltas for the Y values in the same way.

- **(5)** If you had to switch spheres during your video, eliminate the spurious data point by striking through it in the "Delta x" and "Delta y" columns (*not* in the original X or Y column) using Apple-minus.

d. Make histograms of the "Delta x" and "Delta y" columns:

- **(1)** For delta x:
  - **(a)** Insert → Additional Graphs → Histogram.
  - **(b)** Double-click on the histogram to select which columns to display, as well as adjust the settings for the bin width and start position.
  - **(c)**

    Calculate the statistical mean and standard deviation of your data set using the 

button.
  - **(d)** mean =
  - **(e)** standard deviation =
  - **(f)** standard error =
  - **(g)** If there is no overall drift, we expect the mean to be zero. How many standard errors away from zero is it?

    If the mean is more than two SE away from zero, you may have had excessive drift and the rest of the analysis will not work very well. Talk to a TF.
  - **(h)** Does the histogram resemble a Gaussian distribution?
  - **(i)**

    Try fitting a Gaussian to it using the 

button and the "PS2 Gaussian" equation. Record the parameters from the fit here:
    - **I.** mean =
    - **II.** std dev =
    - **III.** How do these compare with the mean and standard deviation you calculated directly from the data?
  - **(j)** Paste a copy of your histogram here:

- **(2)** Now repeat for delta y:
  - **(a)** Insert → Additional Graphs → Histogram; display the delta y column.
  - **(b)**

    Calculate the statistical mean and standard deviation of your data set using the 

button.
  - **(c)** mean =
  - **(d)** standard deviation =
  - **(e)** standard error =
  - **(f)** How many standard errors away from zero?
  - **(g)** Does the histogram resemble a Gaussian distribution?
  - **(h)**

    Try fitting a Gaussian to it using the 

button and the "PS2 Gaussian" equation. Record the parameters from the fit here:
    - **I.** mean =
    - **II.** std dev =
  - **(i)** Paste a copy of your histogram here:

e. When you get to this point, let a TF know so that they can gather your data for the whole class to use later. Now would also be a fine time to save your work.

f. Now, we are going to treat our five-minute random walk as 60 shorter random walks, each lasting five seconds. With this new interpretation, each value of Delta x is really just a final position, x, of such a short walk. We are

interested in finding the *diffusion constant* D, but we know that this is related to the standard deviation of the final position by $\sigma^2 = 2Dt$. This is an easier and more reliable way of determining D than actually calculating the mean squared displacement for each walk.

- **(1)** Calculate D. You can do this from your x results and again from your y results. Don't forget to include the units. (Hint: what are the units of σ?)
  - **(a)** D (from x) =
  - **(b)** D (from y) =
- **(2)** Now we'll try to determine the uncertainty in our calculated values of D. Recall that the uncertainty in the standard deviation of a set of N values is:
  uncertainty in $\sigma = \sigma/\sqrt{(2N-2)}$
  - **(a)** Uncertainty in D (from x) =
  - **(b)** Uncertainty in D (from y) =
  - **(c)** Do the two values of D agree or disagree?

- **(3)** We can let the "final" value of D be the average of the values from x and from y.
  - **(a)** D =
  - **(b)** Uncertainty in D =

g. Deduce a value for Boltzmann's constant k_B from the Einstein-Smoluchowski equation.

- **(1)** You'll need to use the measured properties of the microsphere solution. You'll also need to know the temperature in the lab, which is T=298±1 K.
- **(2)** k_B (including correct units) =
- **(3)** Uncertainty =
- **(4)** What is the accepted value of Boltzmann's constant? How does it compare with your measured value (including uncertainty)?

h. Finally, using R = 8.31 J/K mol, calculate Avogadro's number, $N_A = R/k$.

- **(1)** N_A =
- **(2)** Uncertainty =

9. After the TFs have collected data from every group, they will show you some pretty graphs, from which we can also derive a value of D measured by the whole class. (If you get here before the rest of the class has submitted their data, go on ahead and come back to it later.)

a. The slope of the line relating $\langle x^2 \rangle$ to t gives one way of measuring D:

- **(1)** Slope =
- **(2)** D =
- **(3)** Paste a copy of the graph here:

b. The other way is the histogram of all of the Δx values from the whole class:

- **(1)** D =
- **(2)** Uncertainty =
- **(3)** Paste a copy of the histogram here:

10. Suppose that instead of 1-micron spheres, you had observed 2-micron spheres. What diffusion constant would you have measured?

C. Observe the non-Brownian motion of a biological sample.

1. At the sample preparation station, there are also solutions containing biological samples. Make up a microscope slide containing one of these solutions.

2. In Logger Pro, open the Video Capture window and see if you can focus on a biological sample in your slide.

3. What do you notice qualitatively about the motion of these biological samples? How does it differ from the Brownian motion of the microspheres?

4. You can model a bacterium as a sphere roughly 1 micron in diameter. If it were not self-propelled, how far (on

average) would it diffuse in 1 second? (Treat the problem in two dimensions.) In order for self-propulsion to be useful to the bacterium, it must be able to move itself *faster* than diffusion!

VI. Conclusion

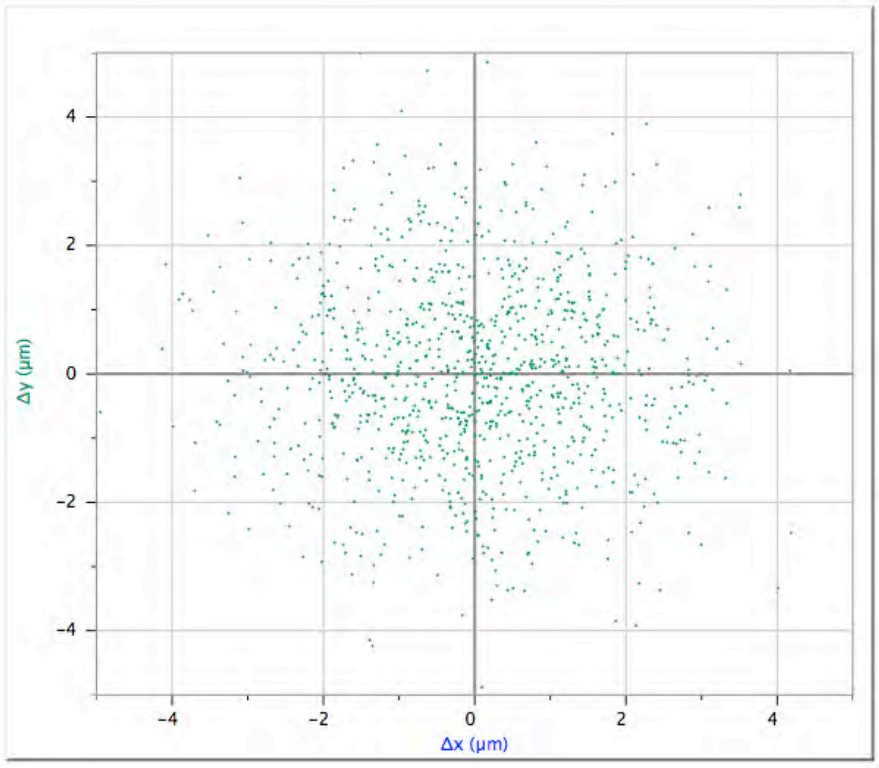
- **A.** Clean-up: place all used slides and cover slips in the jar containing acetone (which will make the cover slips much easier to remove) which is at the sample preparation station. If you have broken glassware or used pipettes, put them in the glass disposal box.
- **B.** Save your work in this file and in Logger Pro. Attach the Logger Pro file here:

- **C.** Submit your work to the physci server using the instructions in the plastic sheath at your computer station. Remember, when you get to the step where you export to HTML, choose "Current Page" instead of "All," and leave the box for cover page **unchecked**.
- **D. Super-duper important—don't even think about skipping this step!** Before you leave the lab, every member of your lab group should open a browser and go to
  http://physci.fas.harvard.edu/~yourFASusername
  and make sure that your lab report is there under the link called "Lab 7." If not, then you haven't submitted it correctly; ask a TF for help.
- **E.** Remember to take your belongings with you when you leave the lab.
- **F.** That's all for this year! We hope you enjoyed the labs and perhaps we'll see you next semester in PS3...

VII. Pre-Lab Assignment

Answer the following questions and bring them with you to lab. Be sure to put your name, your TF's name, and your lab section at the top of your paper.

A. From the PS2 website, download the Logger Pro file for pre-lab 7 and open it on your computer. This file contains the (simulated) results of 1000 two-dimensional random walks, each lasting 10 seconds. You can think of it like this: 1000 identical particles were all released at the origin, and their positions were observed 10 seconds later. Each particle was marked with a dot.



First, make a histogram of the Δx values. What does it look like? What are the mean and standard deviation of the data points? Can you fit a Gaussian to it? Print out a copy of your histogram and attach it.

B. From your histogram, determine the mean squared displacement $\langle(\Delta x)^2\rangle$. (Hint: what does the standard deviation equal if the mean is zero?) Use this to calculate the diffusion constant D.

C. Qualitatively and quantitatively, what would the y-vs-x plot look like if we had let the random walk continue for 20 seconds instead of 10?